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BOTANICAL GAZETTE

SEPTEMBER, 1900

ORIGIN OF THE CONES OF THE MULTIPOLAR SPINDLE IN GLADIOLUS.

ANSTRUTHER A. LAWSON.

(WITH PLATE XII)

RECENT investigation in cytology has gone far to establish the fact that multipolar spindles are of very general occurrence in the higher plants. It is indeed held by many investigators that the origin and development of the karyokinetic spindle in these plants is diametrically opposed to that which prevails in the lower plants and animals. These observers believe that the spindle, instead of being bipolar from the first and under the control of a kinetic center, passes through a series of multipolar stages in the course of its development. The problem has thus become exceedingly interesting and important, and calls for vigorous and careful investigation.

Multipolar spindles have been found in *Lilium* by Farmer (1893), in *Larix* by Belajeff (1894) and Strasburger (1896), in *Equisetum* by Osterhout (1897), in *Lilium*, *Fritillaria*, *Helleborus*, *Podophyllum*, and *Pinus* by Mottier (1897, *a* and *b*), in *Hemerocallis* by Juel (1897), in *Chara* by Debski (1897), in *Zamia* by Webber (1898), in *Sagittaria* by Schaffner (1898), in *Nymphaea* and *Nuphar* by Guignard (1898), in *Hesperaloe*, *Hedera*, *Disporum*, *Smilacina*, *Gladiolus*, *Iris*, and *Cobaea* by Lawson (1898), in *Passiflora* by Williams (1899), in *Solanum* by Nemec (1899), and in *Convallaria* and *Potamogeton* by Wiegand (1899).

Occurring in such a wide range of forms, and having been demonstrated by various methods of fixing and staining, it seems extremely improbable that the phenomenon is abnormal or due to artifact. Such a conclusion is much strengthened by the fact that in none of the higher plants has the animal type of spindle formation been discovered. While it is true that some investigators (Guignard, Schaffner, Fullmer, etc.) have described and figured granules situated at the poles of the spindle, and thus in some respects resembling centrosomes, in no case have such granules been observed to take an actual part in the formation of the achromatic figure as it occurs in animals.

Assuming then that the multipolar type is the normal type of spindle formation in the higher plants, since Strasburger and other authorities cannot reconcile the function of a kinetic center with such a process, we are confronted with two problems, namely, the manner in which the multipolar spindle becomes bipolar, and the origin of the cones.

No explanation has yet been offered that will reasonably account for the ultimate bipolarity of the spindle beyond the mere statement that certain of the cones fuse and arrange themselves in two groups. Such an explanation was first suggested by Belajeff (1894), and was later confirmed by Osterhout (1897), Mottier (1897), and others. How this fusion is brought about is still uncertain. Even Guignard (1898), who describes centrosomes situated at the apices of the cones in *Nymphaea* and *Nuphar*, states that he is "unable to suggest, at present, in what manner the multipolar spindle becomes bipolar." So that with or without centrosomes the phenomenon is unaccounted for, and we must therefore wait for the light of future investigation.

On the origin and development of the cones much more encouraging results have been obtained. The investigations of Belajeff (1894) and Strasburger (1896) on *Larix*, Osterhout (1897) on *Equisetum*, Mottier (1897) on *Lilium*, Juel (1897) on *Hemerocallis*, and more recently those of Nemec on *Solanum* and *Allium*, and Davis on *Anthoceros*, have thrown considerable

light on the subject. But while these observers all agree that the spindle arises from a weft of kinoplasmic threads, very few observations have been made on the very earliest stages of the development of the cones ; probably on account of the fact that these early stages are very difficult to obtain. In 1898, however, the writer was fortunate enough to obtain a very complete series of these early stages in the pollen-mother cells of *Cobaea scandens*. In this case it was found that, as division approaches, the nucleus becomes surrounded by a zone of granular substance which differs in structure and staining properties from the rest of the cytoplasm. This zone is so constant that it was proposed to designate it perikaryoplasm, and as it has been found in several other genera, the writer feels justified in using the term in the present paper. Upon the breaking down of the nuclear wall, the perikaryoplasm forms a central network of kinoplasmic fibers which grows out into several projections. By their growth outward these projections become the cones of the multipolar figure. The spindle fibers are therefore formed by the elongation of the meshes of the net-work in the direction of the projections.

Encouraged by these results the writer was led to pursue his investigation of these early stages in other forms. His observations on *Gladiolus* proved so interesting that it was thought advisable to record them, believing that they will throw further light on this much disputed problem.

The method employed in preparing the material was practically the same as that adopted in my work on *Cobaea*, namely : Flemming's strong solution of chromic-osmic-acetic acid, diluted with one volume of water, was used for fixing, and the triple stain, safranin, gentian-violet and orange G, for staining. I would here like to emphasize the importance of fixing the material in the field, because of all the material I have examined it was only in those anthers which were fixed *immediately* after being detached from the plant that the early multipolar stages were found. After being fixed the anthers were thoroughly washed in running water from six to eight hours, and were then

dehydrated by being passed through various grades of alcohol. Bergamot oil was used to precede the infiltration of paraffin. Microtome sections from $3.6\ \mu$ to $6\ \mu$ thick were used.

THE POLLEN MOTHER CELLS OF GLADIOLUS.¹

Gladiolus affords very exceptional material for the study of spindle formation on account of the large size of its anthers, pollen mother cells, and nuclei. The cytoplasm in the resting pollen mother cell appears in the form of a clear uniform reticulum, with numerous small spherical bodies scattered irregularly through it. The nucleus is very large, containing a vacuolated nucleolus, and the chromatin in a very characteristic spirem. As division approaches, the cytoplasm undergoes a remarkable differentiation in identically the same manner that occurs at this stage in the corresponding mother cells of *Cobaea*. While the chromosomes are being formed, there gradually accumulates a complete and sharply differentiated zone of granular substance about the nucleus. In nearly every respect this zone resembles the perikaryoplasm so characteristic of *Cobaea*. It accumulates in the same manner, has the same structure, and stains in the same fashion. In fact, the only difference that could be detected was in the size of the granules of which the zone is composed. In *Gladiolus* these granules are very fine, while those in *Cobaea* are comparatively coarse. We shall therefore continue to use the term perikaryoplasm in the following description.

By the time the chromatin thread breaks up and assumes the form of curved rod-shaped chromosomes, the perikaryoplasm has reached its maximum development. In many cases it was observed that the small black spherical bodies which were scattered irregularly through the cytoplasm had arranged themselves in the form of a ring at the outer margin of the perikaryoplasm. But this peculiarity is not as constant or as striking as it is in *Cobaea*.

At this early stage in *Cobaea* it was observed that the nuclear membrane breaks down, but in *Gladiolus* this does not happen

¹ One of the common cultivated garden forms, probably of the hybrid *G. Gandavensis* (Hort.).

until a much later stage, as can be seen readily in *figs. 1-8*. This fact is significant, because it is quite clear that the method of spindle development depends much upon *when* the nuclear membrane breaks down. This is very well illustrated by the two types which we have before us. In *Cobaea*, where the nuclear wall breaks down at a very early stage, we have a central network formed which occupies the space of the nuclear cavity. In *Gladiolus*, where the nuclear wall persists, the network is not formed in this central position, but is formed outside of and immediately surrounding the membrane. This difference also has its effect on the character of the network. When the nuclear wall breaks down at an early stage the nuclear cavity affords a large space for the formation of the network. Its meshes are consequently quite large. On the other hand, when the nuclear wall persists, as it does in *Gladiolus*, the network appears in the form of a close weft or felted zone.

Very careful observations were made at this stage to see if any of the kinoplasmic threads penetrate the nuclear membrane, as recently observed by Wiegand in *Potamogeton*. Although hundreds of cells were examined no such penetration was detected.

When this weft or felted zone commences to form, one might at first think that it arose by the fraying out of the nuclear membrane. But as it grows to such a width (*fig. 1*), and the nuclear membrane apparently loses none of its distinctness (*figs. 1-8*), it seems much more probable that it grows at the expense of perikaryoplasm as the central network does in *Cobaea*.

From the time of its first appearance the felted zone stains very readily with gentian-violet, and as it increases in size its meshes become larger and the sharp kinoplasmic threads of which it is composed are readily distinguished. As soon as the weft reaches a certain size (*fig. 1*), it ceases to grow uniformly, but pushes out into several projections as shown in *fig. 2*. As far as the writer could make out, there appears to be no definite number of these projections. *Fig. 2* shows at least five. Six was the largest number observed in cross-section. Just how

many there are it is impossible to say at present. These projections rapidly increase, and on account of their growth outward the meshes become decidedly elongated. By the time they have reached their full growth, the whole outer portions of the cones are composed of long distinct fibers converging to the apices, and it is only at the base that the meshes of the original weft can be distinguished. The apices of the cones taper out into remarkably sharp points, but in no case was there a body observed at these points which might be considered as a controlling center.

During the entire process of the formation of the cones the nuclear membrane remains intact. It is only after they have reached their full development that it begins to break down. The breaking down of the membrane is shown in *figs. 6 and 7*. It will be seen from these figures that this takes place on one side first, where the identity of the membrane becomes lost in the network at the base of the cones. It will also be observed from these figures that the nucleolus still persists. It remains quite conspicuous until all traces of the nuclear wall are lost, when it suddenly disappears. What eventually becomes of it was not observed.

In my observations on *Cobaea* it was thought probable that the linin of the nucleus took part in the formation of the central network from which the cones develop. Now in *Gladiolus*, where the kinoplasmic network and the cones are fully developed before the nuclear wall disappears, it becomes quite evident that the linin takes no essential part in the formation of the achromatic figure.

Upon the disappearance of the nuclear membrane the bases of the cones soon adjust themselves to the space offered by the nuclear cavity, and in doing so come in direct contact, for the first time, with the chromosomes. These latter bodies are not lacking in interest; but as the writer, in future work, hopes to make a more detailed study of the chromatin in *Gladiolus* and other forms, his observations will not be recorded at present. Soon after this stage a series was observed showing that the

cones first approach each other and finally unite in two groups in the form of a bipolar spindle, as shown in *fig. 10*.

From the above observations, and from previous work on other forms, it would seem that there are several types of spindle development in the higher plants. It would be imprudent to classify all of these probable types until the early stages of many of the forms have been more thoroughly investigated. At present we have at least three forms which have been thoroughly worked out and which differ from one another sufficiently to warrant us in distinguishing them as types. These are represented by *Equisetum*, *Cobaea*, and *Gladiolus*.

SUMMARY.

The above observations may be summarized as follows :

As nuclear division approaches, a granular zone accumulates about the nucleus. This zone in every respect resembles the perikaryoplasm so characteristic of the pollen mother cells of *Cobaea*.

A close network or felted zone of kinoplasmic fibers is formed immediately outside of and completely surrounding the nuclear wall. This is probably developed from the perikaryoplasm.

This network grows out into several projections which become the cones of the multipolar figure.

The nuclear membrane persists until the cones are almost fully developed.

The spindle fibers are formed by the elongation of the meshes of the network composing the cones.

Neither the nuclear wall, nucleolus, nor linin take any essential part in the formation of the achromatic figure.

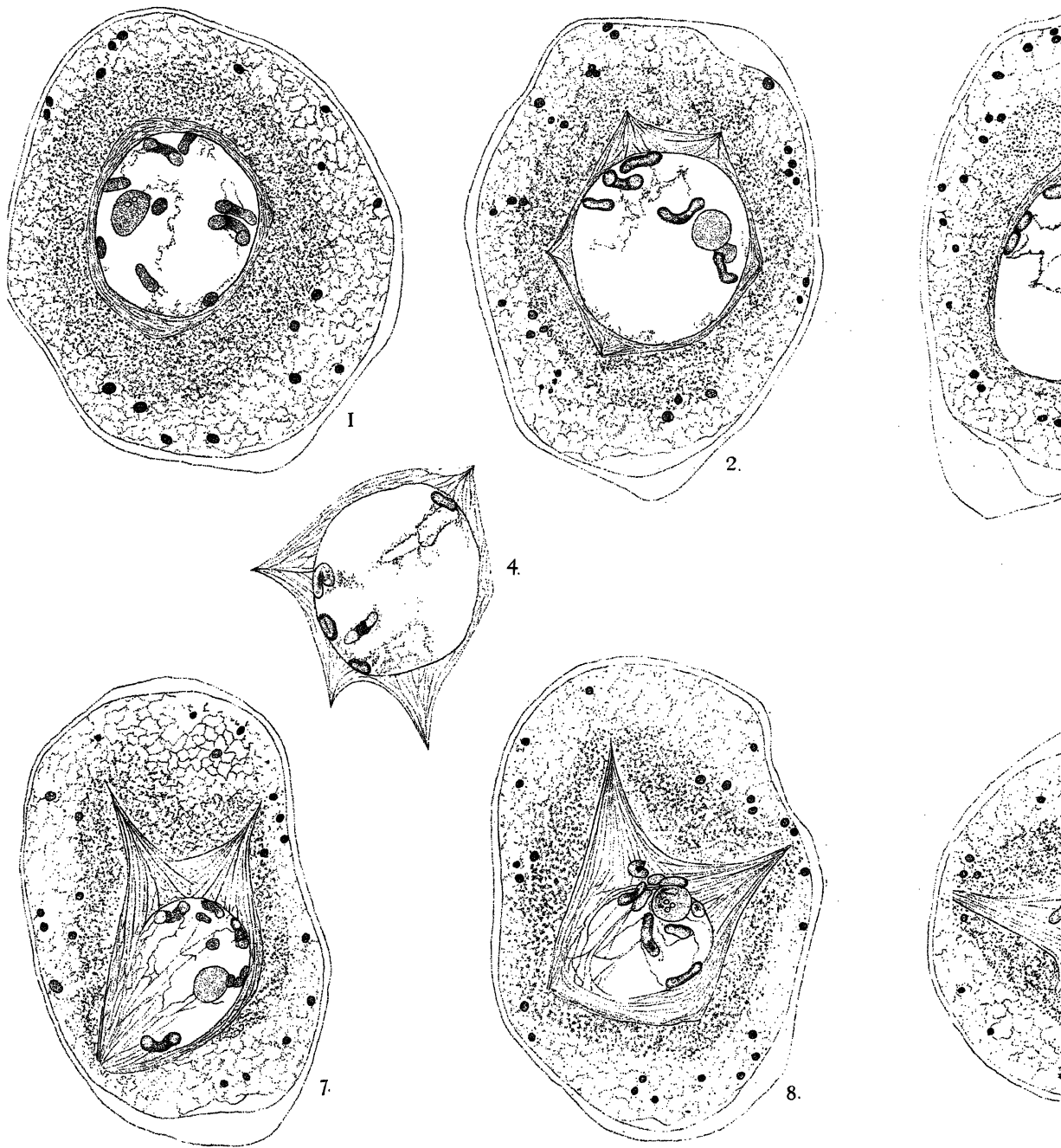
The cones of the multipolar figure fuse and arrange themselves in two groups and form a bipolar spindle.

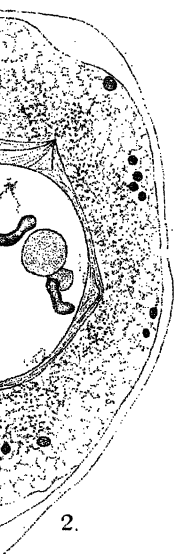
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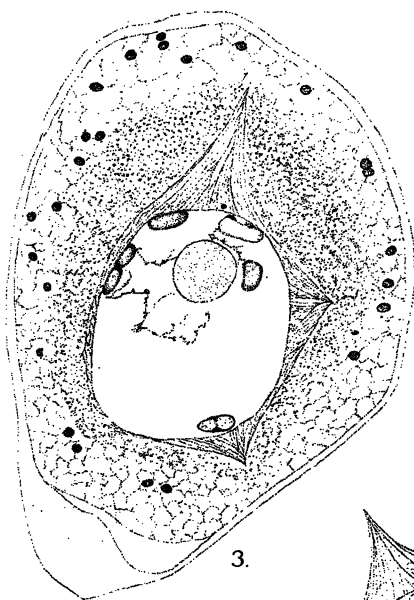
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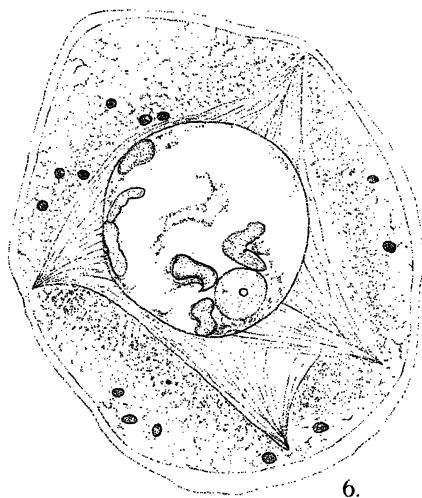




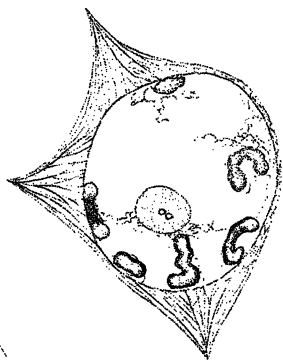
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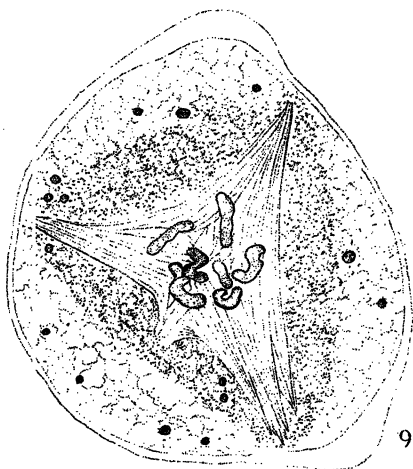
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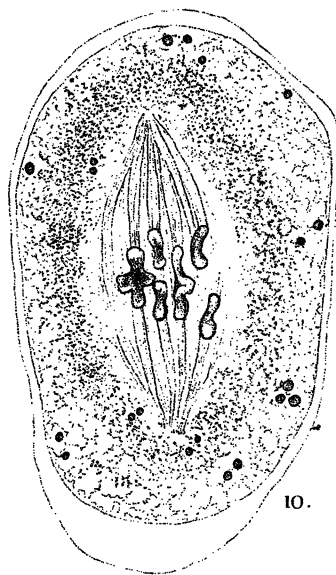
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10.

EXPLANATION OF PLATE XII.

Figures drawn with Abbe's camera lucida, Zeiss homog. immersion objective one twelfth, apert. 1.25, compensating ocular no. 6.

FIG. 1. A pollen mother cell showing the cytoplasm differentiated into three zones; the outer cytoplasm stains a light gray-blue; the perikaryoplasm stains a light orange, and the weft or felted zone of kinoplasmic fibers stains blue; the nuclear wall is intact and the nucleus contains a large nucleolus and several curved chromosomes, with a small amount of linin threads.

FIG. 2. The weft of kinoplasmic threads is commencing to push out into several projections, preparatory to forming the cones of the multipolar figure; the network of the weft appears more distinctly.

FIG. 3. A still later stage in the development of the projection, with distinct cones formed.

FIG. 4. A still later stage showing the elongation of the meshes and the formation of fibers by the pulling out of the network.

FIG. 5. A slightly older stage than that of *fig. 4*.

FIG. 6. A later stage when the cones are nearly fully developed; the outer portions of the cones consist now of distinct fibers, and it is only at the base of the cones that the network of the original weft can be distinguished; the nuclear wall has as yet shown no sign of breaking down, and the nucleolus still persists.

FIG. 7. The first indication of the nuclear wall breaking down. The chromosomes now come in contact with the base of the cones.

FIG. 8. Shows the same as *fig. 7*, but a little more advanced.

FIG. 9. The nuclear wall and the nucleolus have disappeared; several of the cones have fused, and the chromosomes are attached to the fibers at the base of the cones.

FIG. 10. The mature spindle, with the chromosomes at the equator and about to move to the poles.